

**THE EFFECTS OF AQUEOUS *Andrographis paniculata* EXTRACT ON LIPASE,
GLUTATHIONE PEROXIDASE, AND GAMMA-GLUTAMYL TRANSFERASE IN
WISTAR RATS.**

BY

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MAT. NO: LSC 1906519

**RESEARCH PROJECT SUBMITTED TO
THE DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCE
UNIVERSITY OF BENIN
BENIN CITY**

SEPTEMBER, 2023

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IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THR AWARD

BACHELOR OF SCIENCE (B.Sc.) DEGREE IN

BIOCHEMISTRY

SEPTEMBER, 2023

CERTIFICATION

We the undersigned, certify that **IGHODARO BENJAMIN EFOSA** with matriculation number LSC1906519 carried out, compiled and reported this project work in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc, Hons) degree in Biochemistry, in the Department of Biochemistry.

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DEDICATION

This project is dedicated to God Almighty, for all his love, mercy, blessings, and for keeping me alive all through my years in the school. I also dedicate this work to my Parents for their love, financial support and words of encouragement.

ACKNOWLEDGEMENTS

My deepest gratitude goes to God for every help gratitude to me the led to the completion of this projection. Next to him, are my parents, Mr and Mrs IGHODARO BENJAMIN, for the support which was given to me monetarily and through words that inspired the strength needed for this work. I am sincerely grateful to my everworthy project supervisor, Prof. P.O UADIA for every from of guidance that led to the successful completion of this work. I would like to express my deepest appreciation to my project coordinator, Dr. SI Ojeaburu, my head of department, Prof. Oyeneke, and all my lectures, from whom attained knowledge was needed imbibed in me. I would like to extend my gratitude to my parent for their valuable words of encouragement. Last but not the least, my thanks go to my siblings, friends and course mates and relatives who have always been helping and encouraging me.

TABLE OF CONTENTS

CERTIFICATION	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABSTRACT	x
CHAPTER ONE	1
INTRODUCTION AND LITERATURE REVIEW	1
1.1 Introduction	1
1.1.1 STATEMENT OF PROBLEM	2
1.1.1 AIM AND OBJECTIVES	2
1.1.1 SPECIFIC OBJECTIVES OF THE STUDY	2
1.2 LITERATURE REVIEW	2
1.2.1 SCIENTIFIC CLASSIFICATION OF PLANT SPECIES	2
1.2.2 NOMENCLATURE	3
1.2.3 DESCRIPTION	4
1.2.4 HABITAT	4
1.2.5 ECOLOGICAL CONDITION	4
1.2.6 CULTIVATION	5
1.2.7 BIOACTIVE PHYTOCONTITUENTS	6
1.2.7.1 TRADITIONAL USE	7
1.2.7.2 CONTEMPORARY USE	8
1.2.7.3 ANTIBIOTIC ACTIVITY	8
1.2.7.4 NEGATIVE HEALTH IMPACT	9
CHAPTER TWO	10
MATERIALS AND METHODS	10
2.1 MATERIALS	10
2.1.1 EQUIPMENT	10
2.1.2 CHEMICALS AND REAGENTS	11
2.1.3 EXPERIMENTAL ANIMALS	11
2.1.4 ANIMAL FEED	11
2.1.5 ACCLIMATIZATION TREATMENT	12
2.1.6 PLANT SAMPLE AND IDENTIFICATION	12
2.2 METHODS	12

2.2.1 THE AQUEOUS EXTRACT OF <i>A. paniculata</i> WAS PREPARED BY THE FOLLOWING METHOD	12
2.2.2 ACCLIMATIZATION OF ANIMALS	13
2.2.3 INDUCING DIABETES USING STREPTOZOTOCIN	13
2.2.4 TREATMENT OF ANIMALS	14
2.2.5 ANIMAL SACRIFICE AND SAMPLE COLLECTION	14
2.2.5.1 PREPARATION OF SERA SAMPLES	14
2.2.5.2 PREPARATION OF TISSUE HOMOGENATE SUPERNATANT	14
2.2.6 THE LEVEL OF FASTING BLOOD GLUCOSE WAS DETERMINED USING THE FOLLOWING PROTOCOL	15
2.2.7 DETERMINATION OF BODY WEIGHT	15
2.2.8 MORTALITY	16
2.2.9 BIOCHEMICAL ASSAYS	16
2.2.9.1 DETERMINATION OF LIPASE FROM SERUM OR PLASMA	16
2.2.9.2 DETERMINATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY	18
2.2.9.3 DETERMINATION OF Y-GLUTAMYL TRANSFERASE IN SERUM AND PLASMA	19
2.2.9 STATISTICAL ANALYSIS	21
CHAPTER THREE	22
RESULTS	22
3.1 WEIGHT OF RATS	22
3.2 CALCULATION FOR % INCREASE OR DECREASE IN WEIGHT AFTER 2 WEEKS	24
3.3 WEIGHT OF ORGANS	25
3.4 MORTALITY	26
3.5 EFFECT OF AQUEOUS EXTRACT OF <i>Andrographis paniculata</i> ON THE ACTIVITY OF LIPASE IN DIABETIC ANIMALS AFTER 2 WEEKS	26
3.6 EFFECT OF AQUEOUS EXTRACT OF <i>ANDROGRAPHIS PANICULATA</i> ON THE ACTIVITY OF GGT IN DIABETIC ANIMALS AFTER 2 WEEKS	27
3.7 EFFECT OF AQUEOUS EXTRACT OF <i>Andrographis paniculata</i> ON THE ACTIVITY OF GPx IN DIABETIC ANIMALS AFTER 2 WEEKS	27
CHAPTER FOUR	29
DISCUSSION AND CONCLUSION	29
4.1 DISCUSSION	29
4.2 CONCLUSION	31
REFERENCES	32
APPENDIX	37

LIST OF TABLES

Table 3.1: Comparative weight of normal control rat after 2 weeks	22
Table 3.2: Comparative weight of diabetic untreated rats after 2 weeks	23
Table 3.3: Comparative weight of diabetic treated after 2 weeks	23
Table 3.4: % Increase % Decrease in weight after 2 weeks	24
Table 3.5: Weight of organs	25
Table 3.6: Mortality	26
Table 3.7 Activity of lipase after 2 weeks	26
Table 3.8 Activity of GGT after 2 weeks	27
Table 3.9 Activity of GPx after 2 weeks	27

LIST OF FIGURES

Figure 1.1 Image of the Structure of bioactive phytoconstituents of *Andrographis paniculata* 7

ABSTRACT

Andrographis paniculata can be found in different environment ranging from plains, hill, slopes or even road sides and it is grown annually mostly in western asia. It is widely used in the treatment of disease such as sore throat, liver disease, gastrointestinal infection etc. It is characterized by it's bitter taste which is known to contribute to its name " The king of bitters". The aim of this research was to observed and determine the effect of aqueous extract of *Adrographic paniculata* on the activity of lipase, GGT and GPx on streptozotocin induced rat. The results of this study showed that *Andrographis paniculata* has potent therapeutic potential and could be used in the treatment of diabetes.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

A branching, annual plant with lance-shaped green leaves that grows to a height of 60 to 70 cm, *Andrographis paniculata* is an example of this (Mishra *et al.*, 2007). Kabeeruddin and Kitabul (1937) wrote in their journal that in Asian nations including India, Sri Lanka, Pakistan, Java, Malaysia, and Indonesia are among those where it thrives frugally. The name Kalmegh is the most popular one in India. One of the widely employed medicinal herbs in the country, and it may be found in the plains. Additionally, the plant is referred to as the “king of bitters” (Kabeeruddin and Kitabul,1937; Shahid, 2011). Since the entire plant, including the leaves, has a very bitter flavor. It has a long history of medicinal use in conventional Chinese, Indian, and Ayurvedic medicine, as well as Western medicine (Sivananthan and Elamaran, 2013; Pawar *et al.*, 2016; Liu *et al.*, 2016)

A.paniculata is frequently prescribed in Ayurveda to treat gonorrhoea, malaria, dermatitis, intestinal worm infestation, jaundice, sore throat, fever, liver diseases, and jaundice (Pawar *et al.*, 2016; Thokchom *et al.*, 2018) . While treating diarrhoea, laryngitis, gastrointestinal infections, and inflammation, the plant is also used in Chinese medicine to eliminate body heat from the body, including that caused by fevers and toxins (Hu *et al.*, 2017; Yang *et al.*, 2020). *A. paniculata* serves as an immune booster according to modern pharmacology anticancer, antibacterial, stimulant, cures myocardial ischemia, pharyngotonsillitis several more (Hidalgo *et al.*, 2013; Dai *et al.*, 2019) including anti-HIV, anti-inflammatory, etc. Additionally, recent research has found a treatment for SARS-CoV-2 using *A. paniculata* is possible (Banerjee *et al.*,2021).

1.1.1 STATEMENT OF PROBLEM

The aerial part of the plant is rich in a number of chemical constituents which have been in use throughout history as a medicinal plants for mankind. This study will help the effect of aqueous *Andrographis paniculata* extract on lipase, glutathione peroxidase, and gamma-glutamyl transferase in wistar rats.

1.1.1 AIM AND OBJECTIVES

To determine the effect of the aqueous extract of *Andrographis paniculata* on the activity of Lipase, GGT, GPx on streptozotocin induced diabetic rat.

1.1.1 SPECIFIC OBJECTIVES OF THE STUDY

To determine the effect of aqueous extract of *Andrographis paniculata* on

- (1) The activity of lipase in diabetic rats
- (2) The activity of GPx in diabetic rats
- (3) The activity of GGT in diabetic rats

1.2 LITERATURE REVIEW

1.2.1 SCIENTIFIC CLASSIFICATION OF PLANT SPECIES

Domain.....Eukaryota

Kingdom.....Plantae

Phylum.....Tracheophyta

Subphylum.....Angiospermae

Class.....Magnoliopsida (Dicotyledoneae)

Order.....Tubittorae

Family.....Acanthaceae

Genus.....Andrographis

Specie..... Paniculata Nees

1.2.2 NOMENCLATURE

Preferred scientific name: *Andrographis Paniculata*

Common name: King of Bitter, -The Creat (Abhishek *et al.*, 2010).

Nigerian names: Yoruba- Meje , Hausa- Garadugu

1.2.3 DESCRIPTION

It is a 0.5-1.0 m tall, erect, annual herb with several branches and a tap root. The leaves are glabrous, lanceolate, 3–7 cm 1-2.3 cm in size, green, with an acuminate apex and a tapering base. Flowers are tiny and solitary; the hairy corolla is either white or pale pink in color. Fruit is a capsule with many seeds that is linear, oblong, and sharp at both ends. While it is commonly farmed in China, Thailand, the East and West Indies, and Mauritius, it grows in South Eastern Asia, specifically in India, Sri Lanka, Pakistan, and Indonesia. In general, it can be found in all types of vegetated regions, including pine, evergreen, and deciduous forest areas, as well as along roads and in settlements. (Abhishek *et al.*, 2010).

1.2.4 HABITAT

There are many different environments where it can be found, including plains, hill slopes, wastelands, farms, dry or wet regions, sea coasts, and even road sides (Abhishek *et al.*, 2010).

1.2.5 ECOLOGICAL CONDITION

It may be grown from seeds on any type of soil with ease (Abhishek *et al.*, 2010).

1.2.6 CULTIVATION

It is grown as a crop during the rainy season (Kharif) in India. For the plant to thrive, the environment must be hot, humid, and sunny. Kalmegh can be grown on a variety of soil types, including lateritic and loam soils with a moderate fertility. Additionally, it can be grown on shaded wastelands. Following the end of the monsoon, the plant begins to flower as it develops luxuriantly with the approach of the next rainstorm. Broken seeds are used for reproduction in nature. Layering can also be used for vegetative propagation in some unique situations because each node can produce enough roots. Small and dormant for 5–6 months, seeds are tiny. Three beds of 10 x 2 m each need to be tilled, ground up, and leveled in order to raise crops on a hectare of land. For developing healthy seedlings, it is advisable to utilize organic manure liberally in the nursery. On each bed surface, 250–300g of seeds are disseminated, and a thin layer of a soil and compost combination should be applied to cover them. Till seedlings sprout (in 6-7 days), the bed should be thoroughly covered with the right kind of mulch and periodically watered by a fountain. To prevent seedling elongation, mulch must be removed as soon as germination (70–80%). To protect them from the heat, seedlings should ideally be nurtured in a shaded area. When seedlings are one month old, they are transplanted into well-prepared and laid-out beds with a row spacing of 45-60 cm and a plant spacing of 30-45 cm. After transplantation, beds need to be watered to maximize the herb output. It can be cultivated on infertile to moderately fertile soil, however increasing the herb yield by applying 80 kg of nitrogen and 40 kg of phosphorus per hectare can help. Two nitrogen doses can be administered: one as a basal dose and the other after 30-45 days of transplanting. For growing nurseries, an additional 3-6 tonnes of well-rotten farmyard manure are needed. In 90 to 100 days, the maximum amount of herb biomass can be harvested before the leaves begin to shed. Throughout

the winter, the crop is dormant. The amount of the active ingredient andrographolide in leaves is high around the period of flower commencement. Since the entire plant contains active ingredients, the harvested material is dried in the shade before being ground up. In the monsoon season, a well-maintained crop yields 3.5 to 4 tonnes/ha of herb (Chauhan and Gyanendra, 2003; Maheshwar *et al*, 2002; Seema *et al.*, 2003).



Plate 1: The leaves of *Andrographis Paniculata*

Source: (Akilandeswari *et al.*, 2019)

1.2.7 BIOACTIVE PHYTOCONTITUENTS

Flavonoids, lactones, and diterpenes are all present in *A. paniculata*. Flavonoids have been separated from the leaves and are primarily found in the roots of plants. There are alkanes, ketones, and aldehydes in the aerial sections. Although it was first believed that the lactone andrographolide was the source of the leaves' bitterness, later research revealed that the leaves

really contained two bitter compounds, andrographolide and a chemical known as kalmegin four lactoneschuanxinlian. In China, the aerial portions were used to isolate the compounds A (deoxyandrographolide), B (andrographolide), C (neoandrographolide), and D (14-deoxy-11,12dihydroandrographolides). Six diterpenoids of the ent-labdane type, including two diterpenes, glucosides, and four diterpene dimmers (bis-andrographolides A, B, C, and D), have been isolated from aerial parts. Diterpene glucoside (deoxyandrographolide 19 beta-d-glucoside) has been found in the leaves (Akilandeswari *et al*; 2019).

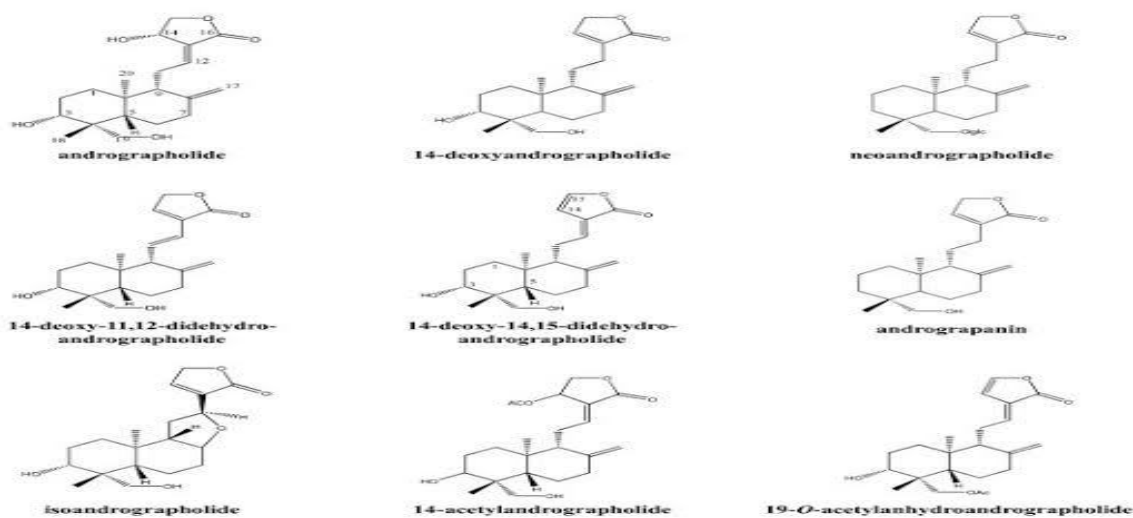


FIG 1.1: IMAGE OF THE STRUCTURE OF BIOACTIVE PHYTOCONSTITUENTS OF *Andrographis paniculata*

1.2.7.1 TRADITIONAL USE

- *A.paniculata* is a traditional medicine that is said to regulate body temperature, improve the body's natural detoxification processes, and protect against common colds, upper respiratory tract infections, sinusitis, and fever (Gabrielian *et al.*, 2002).
- *A. paniculata* is used as a cure for poison caused by snake and insect (Samy *et al.*, 2008).

- The plant may have therapeutic properties that could help treat liver disorders and alleviate the symptoms of common colds and coughs (Geethangili *et al.*, 2008).
- Typical applications Since ancient times, lower urinary tract infections, herpes, fever, sore throats, and respiratory illnesses have all been successfully treated with the traditional medicinal plant *Andrographis paniculata* (Jarukamjorn and Nemoto, 2008)
- *A. paniculata* is sometimes prescribed to treat skin conditions such as leprosy, gonorrhoea, scabies, and boils. Its ‘blood purifying’ properties are also believed to be effective in reducing fever symptoms.

1.2.7.2 CONTEMPORARY USE

Pharmacological and clinical research has indicated that *A. paniculata* may have beneficial effects in the treatment of diseases such as cancer and HIV (Shahid, 2011).

1.2.7.3 ANTIBIOTIC ACTIVITY

The study evaluated the effect of *A. paniculata* in rats with streptozotocin-induced diabetes. The results showed that *A. paniculata* could lower blood glucose levels and improve the health of the rats’ beta cells. The study also found that a specific compound in *A. paniculata*, andrographolide, could improve the function of pancreatic beta cells and increase insulin production. This suggests that *A. paniculata* may be useful in the treatment of diabetes (Nugroho *et al.*, 2013). (Zhang *et al.*, 2012) investigated the effects of andrographolide on type I diabetes in mice. The mice were given water or different doses of andrographolide for four weeks. The mice that received andrographolide had improved glucose tolerance and their pancreatic islets showed reduced damage. There was a decrease in the production of Th1 and Th17 cytokines, such as IFN- γ and IL-17. The production of the Th2 cytokines IL-10 and TGF- β was increased. (Chaurask *et al.*, 2012) studied the anti-diabetic activity of the chloroform fraction of the ethanol extract of *A.*

paniculata in type 2 diabetic mice. The results showed that the fraction significantly improved fasting blood glucose, oral glucose tolerance, serum insulin levels, and tissue glucose content. In addition, the activity of the enzyme glucose-6-phosphatase and hexokinase was evaluated in body tissues.

1.2.7.4 NEGATIVE HEALTH IMPACT

It has been reported that high doses of AP extract may cause nausea, vomiting, dizziness, and allergic reactions. When taken with anticoagulant medications, it may increase the risk of bleeding and bruising, since AP extract inhibits platelet aggregation and blood clotting (Nishran *et al.*, 2014).

CHARTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 EQUIPMENT

- Nose mask
- Mechanical Blender (Cixi Honest, China).
- Bench-top centrifuge (centrifuge 80–20, Techmel & Techmel, USA).
- Spectrophotometer (Bruker Physik, Germany).
- Weighing Balance (Gallenkamp, England).
- Micropipettes [200µl and 1000µl (Sigma, USA)]
- Gavage (Bajaj Instruments, India).
- Cages
- Dissecting kit (Gold Cross, England).
- Ceramic plates
- Muslin Cloth
- Beakers (Pyrex, Nigeria).
- Rotatory Evaporator (US Lab, USA)
- Conical Flasks (Technico, India).
- Cotton wool
- Glucometer and strips (Yasee GLM–79, China).
- Syringes (2ml and 5ml [SeaskyMedical, China]).
- Hand gloves

2.1.2 CHEMICALS AND REAGENTS

All the chemicals used in this experiment were of the highest possible purity. They included the following chemical:

- Sucrose (Chempur, Germany).
- Picric Acid (Dow, USA).
- Malondialdehyde Assay Kit (Randox Lab Ltd, U.K).
- Glutathione Peroxidase Assay Kit (Randox Lab Ltd, U.K).
- Reduced Glutathione Assay Kit (Randox Lab Ltd, U.K).
- Streptozotocin (Sigma, London).
- Chloroform (Sigma–JHD, Germany)
- Sodium Citrate (May and Baker, England)
- Sodium Chloride (Chempur, Germany).

2.1.3 EXPERIMENTAL ANIMALS

Eighteen (18) Male Wistar rats were obtained, weighed, housed in clean cages where they were fed standard diet and water. They were procured from the animal house from the department of Anatomy, University of Benin, Nigeria. On transferred to the Animal house of the University of Benin.

2.1.4 ANIMAL FEED

Grower mash was purchased from Uselu Market in Benin City, Nigeria. The grower mash was part of the Chikun Grower brand, which is a type of pelleted feed commonly used for chickens and other poultry in Nigeria. They were fasted overnight before sacrifice.

2.1.5 ACCLIMATIZATION TREATMENT

The cages and the entire animal house were disinfected prior to the commencement of the study. The animals were given two weeks to acclimatized in the animal house before the commencement of the study.

2.1.6 PLANT SAMPLE AND IDENTIFICATION

The *A. paniculata* plants used in this study were sourced from Benin City, Nigeria, and were then identified as true specimens by Prof. Aigbiokhan Emmanuel Izaka with voucher number UBH-A599 from the Department of Plant Biology and Biotechnology at the University of Benin, Benin City and subsequently deposited in the Herbarium for future reference.

2.2 METHODS

2.2.1 THE AQUEOUS EXTRACT OF *A. paniculata* WAS PREPARED BY THE FOLLOWING METHOD

About 270 grams sample of dried and ground ‘King of bitters’ was weighed using a weighing balance. A sample was poured into a conical flask containing 1350 ml of distilled water. The mixture was stirred and brought to boil. After 10 minutes of boiling, the mixture was allowed to cool. Filtration was carefully carried out using a muslin cloth. The filtrate was poured into an air-tight flask and stored.

2.2.2 ACCLIMATIZATION OF ANIMALS

The experiment began with the purchase of 18 male albino rats from the animal house at the University of Benin. The rats were of varying weights, ranging from 111 to 178 grams, and were divided into 3 groups. Each group was placed in a different galvanized cage and allowed to acclimatize for 7 days, during which time they were given grower pellets and water and libitum. After this acclimatization period, the baseline blood glucose levels of the rats were measured to provide a control value for comparison with subsequent measurements.

2.2.3 INDUCING DIABETES USING STREPTOZOTOCIN

Sodium citrate buffer (0.1M, pH 4.5) was used to dissolve freshly prepared streptozotocin, with a concentration of 300mg per 12ml of buffer solution. The rats were then injected with this solution, using a dose of 50ml/kg body weight.

To observe the effects of streptozotocin, the rats were divided into 3 groups, each with a different number of rats.

1. Normal Control (AC, 5 rats) – Rats were injected with the buffer only and weren't treated with the aqueous extract.
2. Diabetes Untreated (AU, 7 rats) – Rats were injected with STZ only and weren't treated with the aqueous extract.
3. Diabetes Treated (AT, 6 rats) – Rats were injected with STZ and treated with the aqueous extract.

After the injections, the rats were given access to 10% sucrose water for the next 24 hours. The rats' blood glucose levels were measured before the treatment and before sacrifice.

2.2.4 TREATMENT OF ANIMALS

A. paniculata extract of 5g was dissolved in a 100ml beaker of distilled water. The rats in the Diabetes treated group were given 300ml/kg of the aqueous extract orally via gavage every day for 21 days.

2.2.5 ANIMAL SACRIFICE AND SAMPLE COLLECTION

The animals were treated humanely and after the final extract treatment, the animals were sacrificed 24 hours later. Each rat was anesthetized with chloroform, and the thoracic and abdominal regions were opened with a dissection kit. Blood samples were taken by cardiac puncture, while the entire pancreas was excised and stored in a sample bag on ice.

2.2.5.1 PREPARATION OF SERA SAMPLES

The blood samples were centrifuged at 3000rpm for 15 minutes to separate the serum. The serum was then collected using Pasteur pipettes and stored at -20C until required for biochemical analysis.

2.2.5.2 PREPARATION OF TISSUE HOMOGENATE SUPERNATANT

The pancreas was first weighed to determine its mass, and then homogenized in saline to create a suspension of pancreatic tissue. The suspension was then centrifuged at 3000rpm for 15 minutes, after which the clear supernatant containing cellular components was collected and stored in plain bottles at -20°C until further analysis.

2.2.6 THE LEVEL OF FASTING BLOOD GLUCOSE WAS DETERMINED USING THE FOLLOWING PROTOCOL

PRINCIPLE

The glucometer's manual described the method of measuring fasting blood glucose levels as per the protocol of Barham and Trinder (1972). The glucose meter's enzyme-based reaction works by combining the glucose in the patient's blood sample with enzymes in a disposable test strip or reaction cuvette. This reaction produces a color change proportional to the concentration of glucose in the solution, which is measured by the meter and displayed on the screen.

PROCEDURE

After fasting overnight, a drop of blood was collected from the tip of each rat's tail using a needle and a glucometer was used to measure the blood glucose level of each rat. The blood glucose levels were recorded before and after the administration of STZ, and at the time of sacrifice. The rats were considered diabetic if their blood glucose levels were above 200mg/dl.

2.2.7 DETERMINATION OF BODY WEIGHT

The body weight of each rat was determined by placing the rat on a weighing balance, following the instructions in the balance's manual. This method was adapted from the work of Tonyushkina and Nichols (1983).

PRINCIPLE

An object placed on a digital balance displaces the strain gauge, which measures the change and converts it into an electrical signal that's passed through a digital converter. The digital converter displays the object's weight on the balance's screen.

PROCEDURE

The initial weight and final weight of each animal were recorded using a weighing balance. The initial weight was measured the day the animals were purchased, and the final weight was measured a day before they were sacrificed.

2.2.8 MORTALITY

Aqueous Control – None of the 5 rats in this group died before the day of sacrifice.

Aqueous Untreated – Four (4) out of the seven (7) rats died before the day of sacrifice.

Aqueous Treated – Two (2) out of the seven (6) rats died before the day of sacrifice.

2.2.9 BIOCHEMICAL ASSAYS

2.2.9.1 DETERMINATION OF LIPASE FROM SERUM OR PLASMA

TEST PRINCIPLE

Lipases are glycoproteins with a molecular weight of 47000 daltons. They are triglyceride hydrolases that catalyzed the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. The determination of lipase is central for differential diagnosis of diseases of the pancreas. The method described here is based on the cleavage of a specific chromogenic lipase substrate 1, 2, o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester emulsified with bile acids. The combination of bile acid and colipase is

used in this assay to detect the lipase activity. In the assay, the rate of formation of methylresorufin is measured photometrically and is directly proportional to the activity of lipase.

MANUAL PROCEEDURE

Wavelength	Temperature	Cuvette	Measurement
580nm (578nm)	37°C	1cm light path	Against Distilled water

Pipette into tubes as follows (37 ^o C)		
	Blank	Calibrator
R1 Buffer	1000µl	1000µl
R2 Buffer	500µl	500µl
Distilled water	30µl	_____

Mix and incubate at assay temperature for 1 minute, Measure initial absorbance of the sample, start the stopwatch at the same time.

Measure absorbance at 1.2 minutes. Calculate $\Delta\text{Abs}/\text{minute}$.

Calculation:

$\Delta\text{Abs}/\text{min}$ of Calibrator = (ΔAbs Calibrator - ΔAbs Blank)

$\Delta\text{Abs}/\text{min}$ of Sample = (ΔAbs Sample - ΔAbs Blank)

Lipase activity = $\Delta\text{Abs} / \text{min of Sample} \div \text{Abs/ min of Calibrator} \times \text{Calibrator value}$

QUALITY CONTROL

It is recommended that a laboratory uses normal and abnormal reference Control sera to verify the performance of the procedure, both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.

2.2.9.2 DETERMINATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY

Glutathione peroxidase was estimated by the method described by Nyman (1959).

PRINCIPLE

This is based on the oxidation of pyrogallol to purpuragallin by peroxidase, resulting to a light brown colouration, which was read at 430nm.

PROCEDURE

To 0.2ml of serum, 2.5ml of phosphate buffer (pH 7.4), 2.5ml of H₂O₂ and 1.5ml of pyrogallol were added. The reaction was allowed to stand for 30min at room temperature. The colour developed was read at 430nm.

Calculation:

Enzyme activity = $\frac{\text{OD/min} \times \text{vt}}{\text{U/mg protein}}$

$$E \times V_s \times Y$$

Where:

OD = absorbance

V_t = total volume of reaction mixture

E = Molar extinction co-efficient

V_s = volume of sample

Y = mg of protein used

2.2.9.3 DETERMINATION OF Y-GLUTAMYL TRANSFERASE IN SERUM AND PLASMA

TEST PRINCIPLE

The substrate L-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted to 5-amino-2-nitrobenzoate by Y-GT measured at 405nm. The increase in absorbance is proportional to y-GT activity.

L-y-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{Y-GT}}$

L-Y-glutamylglycylglycine + 5-amino- 2- nitrobenzoate.

MANUAL PROCEDURE:

Wavelength	Temperature	Cuvette	Measurement
Hg 405 nm (400-420 nm)	25, 30 and 37°C	1 cm light path	Against Air or Distilled water

PROCEDURE 1: SAMPLE START

Pipette into a cuvette as follows:		
	Blank	Sample
Sample	-	1000 μ l
Working Reagent	1000 μ l	1000 μ l
Mix well and allow to stand for 1 min at 25°C (30 or 37°C)		
Measure the absorbance of the sample per minutes for 3 minutes. (Δ Abs/min)		

PROCEDURE 2: SUBSTRATE START

Pipette into cuvette as follows:		
	Blank	Sample
R1 Buffer/Glylglycine	1000 μ l	1000 μ l
Sample		100 μ l
Mix well and allow to stand for 3 minutes at 37°C. Add:		
R2 Substrate	200 μ l	200 μ l
Mix well and allow to stand for 2 minutes at 37°C, Measure the absorbance of the sample per minute for 4 minutes. (Δ Abs/ min)		

CALCULATION:

To calculate the GGT activity, use the following formula:

$U/L = 1158 \times \Delta A_{405} \text{ nm/min}$ for procedure 1. (Sample start)

$U/L = 1368 \times \Delta A_{405} \text{ nm/min}$ for procedure 2. (Substrate start)

2.2.9 STATISTICAL ANALYSIS

The results are presented as mean \pm SEM. They were analyzed statistically using one-way Analysis of Variance test (ANOVA) SPSS version 17 and the significance of differences between mean values was determined using least square difference (LSD). They were considered significant at p-value < 0.05

CHAPTER THREE

RESULTS

3.1 WEIGHT OF RATS

Table 3.1 gives a detailed look at how the weights of animals changed during a diabetic research study. “Basal weight” is the starting weight when we got the animals. “Weight at Day 0” is their weight when we induced diabetes using STZ “Day 7” is their weight one week after treating them with an extract from *Andrographis paniculata*. “Day 14” is their weight when we analyzed them after two weeks.

TABLE 3.1 COMPARATIVE WEIGHT OF NORMAL CONTROL RATS AFTER 2 WEEKS

Group	1	Basal weight (g)	Wt. of animals after acclimatization- Day 0 (g)	Wt. of animals on the 8th day of treatment- Day 7 (g)	Wt. of animals on the 14th day of treatment- Day 14 (g)
R1		148.01	190.51	134.28	256.78
R2		144.36	182.42	222.80	245.06
R3		146.10	181.03	216.45	244.02
R4		143.77	169.05	190.43	208.05
R5		146.44	181.24	212.65	229.71

TABLE 3.2 COMPARATIVE WEIGHT OF DIABETIC UNTREATED RATS AFTER 2 WEEKS

Group	2	Basal weight (g)	Wt. of animals after acclimatization- Day 0 (g)	Wt. of animals on the 8th day of treatment- Day 7 (g)	Wt. of animals on the 14th day of treatment- Day 14 (g)
(Diabetic untreated)					
R1		171.46	195.69	144.42	144.76
R2		172.43	197.96	159.74	153.04
R3		160.37	179.51	220.35	239.27

TABLE 3.3 COMPARATIVE WEIGHT OF DIABETIC TREATED RATS AFTER 2 WEEKS

Group	3	Basal weight (g)	Wt. of animals after acclimatization- Day 0 (g)	Wt. of animals on the 8th day of treatment- Day 7 (g)	Wt. of animals on the 14th day of treatment- Day 14 (g)
(Diabetic treated with aqueous extract)					
R1		150.45	174.46	137.42	126.55
R2		156.13	184.75	172.83	152.63
R3		151.99	186.69	146.02	132.67
R4		157.82	161.86	132.95	124.02

3.2 CALCULATION FOR % INCREASE OR DECREASE IN WEIGHT AFTER 2 WEEKS

Initial Weight – Weight at Day 0

Final Weight – Weight at Day 14

$$\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times \frac{100}{1}$$

TABLE 3.4 % INCREASE % DECREASE IN WEIGHT

Group 1 (Normal control)	% Increase % Decrease in weight
R1	36.33 %
R2	45.31%
R3	40.39%
R4	26.91%
R5	37.39%
Group 1 (Diabetic untreated)	% Increase % Decrease in weight
R1	-23.72%
R2	-10.70%
R3	-13.49%
R4	10.65%
Group 3 (Diabetic treated with aqueos extract)	% Increase % Decrease in weight

R1	-19.17%
R2	-17.45%
R3	-19.49%

3.3 WEIGHT OF ORGANS

TABLE 3.5 WEIGHT OF ORGANS

Group 1 (Normal control)	Wt. of liver (g)	Wt. of pancreas (g)
R1	7.420	0.640
R2	5.020	0.575
R3	7.630	0.569
R4	8.839	0.720
R5	5.820	0.640
Group 2 (Diabetic untreated)	Wt. of liver (g)	Wt. of pancreas (g)
R1		0.230
R2		0.515
R3		0.260
R4		0.560
Group 3 (Diabetic treated with aqueous extract)	Wt. of liver (g)	Wt. of pancreas (g)
R1	5.475	0.200
R2	5.230	0.200

R3	4.554	0.300
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3.4 MORTALITY

TABLE 3.6 MORTALITY

Group 1 (Normal control)	Nil
Group 2 (Diabetic untreated)	3
Group 3 (Diabetic treated with aqueous extract)	3

3.5 EFFECT OF AQUEOUS EXTRACT OF *Andrographis paniculata* ON THE ACTIVITY OF LIPASE IN DIABETIC ANIMALS AFTER 2 WEEKS

TABLE 3.7 ACTIVITY OF LIPASE AFTER 2 WEEKS

GROUPS	MEAN±STD (U/L)
NORMAL CONTROL	268±27.7 ^a
DIABETIC CONTROL	124±42.6 ^b
DIABETIC TREATED	100±6.93 ^c

All values are expressed as mean ± standard deviation (n=3)

Values on the same column with the same alphabets do not differ significantly ($p>0.05$) while data with different superscripts are significantly different ($p<0.05$).

3.6 EFFECT OF AQUEOUS EXTRACT OF *ANDROGRAPHIS PANICULATA* ON THE ACTIVITY OF GGT IN DIABETIC ANIMALS AFTER 2 WEEKS

TABLE 3.8 ACTIVITY OF GGT AFTER 2 WEEKS

GROUPS	MEAN±STD (U/L)
NORMAL CONTROL	1.93 ±1.34 ^a
DIABETIC CONTROL	7.69±0.64 ^b
DIABETIC TREATED	5.79±1.16 ^c

All values are expressed as mean ± standard deviation (n=3)

Values on the same column with the same alphabets do not differ significantly ($p>0.05$) while data with different superscripts are significantly different ($p<0.05$).

3.7 EFFECT OF AQUEOUS EXTRACT OF *Andrographis paniculata* ON THE ACTIVITY OF GP_x IN DIABETIC ANIMALS AFTER 2 WEEKS

TABLE 3.9 ACTIVITY OF GP_x AFTER 2 WEEKS

GROUPS	MEAN±STD (U/mL)
NORMAL CONTROL	35.6±5.3 ^a

DIABETIC CONTROL	35.6±6.1 ^a
DIABETIC TREATED	26.1±3.9 ^b

All values are expressed as mean ± standard deviation (n=3)

Values on the same column with the same alphabets do not differ significantly ($p>0.05$) while data with different superscripts are significantly different ($p<0.05$).

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

The therapeutic potential of *Andrographis paniculata* was evaluated using its aqueous extract to treat streptozotocin induced diabetic rats. Lipase, GGT and GPx assays were conducted to study the effect of the extract on these markers. The results of the study showed that *Andrographis paniculata* has therapeutic potential and could be used in the treatment of diabetes.

During the period of time when these wistar rats were induced with STR resulting in them being diabetic, it was observed that they became restless, defecating and peeing more than usual.

ACTIVITY OF LIPASE

Comparing Normal Control to Diabetes Control, inducing STZ reduced lipase activity (albeit not significant; $p > 0.05$) in the aqueous groups. Comparing Normal Control to Diabetes Treated, treatment with the aqueous extract significantly decrease the lipase activity ($p > 0.05$), which helped to reverse the diabetic effects of STZ (Diabetic Control/ Diabetic Treated).

According to Daniel *et al.*, (2022), he contributed to this claimed by stating Diabetes can lead to changes in the levels of lipids (fats) in the blood. This can cause a condition called dyslipidemia, which is a risk factor for developing atherosclerosis (narrowing and hardening of the arteries). One reason this happens is that the levels of hormones that regulate fat metabolism are out of balance. Normally, the hormone insulin helps to activate an enzyme called lipoprotein lipase, which breaks down triglycerides (a type of fat). In diabetes, though, insulin levels are low, so lipoprotein lipase isn't activated and triglyceride levels can become too high. This, in turn, can cause cholesterol levels to become elevated, leading to metabolic abnormalities. In diabetes, low

levels of insulin can lead to a buildup of triglycerides in the blood. High triglyceride levels reduce HDL cholesterol ("good" cholesterol) and increase LDL cholesterol ("bad" cholesterol). A major feature of diabetes is a change in the level of blood lipids, with higher levels of triglycerides, total cholesterol, and LDL cholesterol and lower levels of HDL cholesterol. Treatment with the extract and fractions of *N. canescens* reversed this effect and restored blood lipid levels to normal levels.

ACTIVITY OF GGT

In contrast to lipase activity, the STZ increased GGT activity levels (significant; $p < 0.05$) in the aqueous groups (Normal Control / Diabetic Untreated). Treatment with the aqueous extract equally did slightly reverse this effect (Diabetic Control/ Diabetic Treated), as GGT levels of the Diabetic Treated rats were significantly lower ($p < 0.05$) than in Normal Control rats (Normal Control/ Diabetic Treated).

This was in contrast to the work of Akuru, et al (2019), For GGT, the animals treated with 500mg/kg of *B. owariensis* had significantly lower activity ($p \leq 0.05$) compared to control and DMBA-treated animals. *E. polysperma* treatment also reduced GGT activity, but the difference was not significant. High levels of GGT in the blood are usually associated with oxidative stress, especially during carcinogenesis. The findings suggest that the extracts may have reduced the oxidative stress caused by DMBA.

ACTIVITY OF GPx

Relative to Normal Control rats, inducing STR into the rat on comparison to Diabetic Control shows absolutely no changes in the GPx activity level ($p > 0.05$). However, when compared to Diabetic Treated it appears that the GPx activity levels reduced significance, it was also the same for Diabetic Control compared with Diabetic Treated ($p < 0.05$).

This claimed was supported by Sukalingam *et al.*, (2018), his study investigated the effect of an aqueous extract of *J. tranquebariensis* leaves on TAA-induced liver fibrosis in rats. Liver injury can be detected by the release of enzymes from damaged liver cells. The increase in serum enzymes in this study indicated liver injury.

TAA-induced rats showed decreased GPx activity, which might be due to the increase in oxidative stress. In TAA-induced rats that were given the *J. tranquebariensis* extract, GPx activity was restored to levels seen in the control group.

4.2 CONCLUSION

The results of this study suggest that the aqueous extract of *Andrographis paniculata* may be effective in managing streptozotocin induced diabetes, particularly due to its ability to improve lipase activity, GGT and GPx levels. However, further research is needed to understand the mechanism of action and to explore the full potential of this extract for diabetes management. This could lead to more targeted and effective therapeutic application in the future.

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APPENDIX

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Mini mum	Maxi mum
						Lower Bound	Upper Bound		
						LIP ASE	Sample 1		
	Sample 2	3	124.000	42.56759	24.57641	18.2562	229.7438	78.00	162.00
	Sample 3	2	102.000	8.48528	6.0000	25.7628	178.2372	96.00	108.00
	Total	8	172.500	84.16820	29.75795	102.1336	242.8664	78.00	300.00
GPx	Sample 1	5	35.5860	5.25563	2.35039	29.0603	42.1117	28.96	43.32
	Sample 2	3	35.6467	6.15921	3.55602	20.3463	50.9470	28.93	41.03
	Sample 3	4	26.0525	3.98015	1.99007	19.7192	32.3858	20.67	30.10

	Total	12	32.42	6.58797	1.901	28.2375	36.6091	20.67	43.32
			33		78				
GG	Sampl	3	1.930	1.33714	.7720	-1.3916	5.2516	1.16	3.47
T	e 1		0		0				
	Sampl	3	7.722	.67088	.3873	6.0561	9.3892	6.95	8.11
	e 2		7		3				
	Sampl	3	5.790	1.15900	.6691	2.9116	8.6698	4.63	6.95
	e 3		7		5				
	Total	9	5.147	2.72385	.9079	3.0540	7.2415	1.16	8.11
			8		5				

Multiple Comparisons

Dependent Variable	(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
						Lower Bound	Upper Bound	
LIPA	LSD	Sample	Sample	144.00000	26.412	.003	76.1055	211.8945
SE		1	2	*	12			

		Sample 3	Sample 3	166.00000 *	29.529 65	.002	90.0916	241.9084
		Sample 2	Sample 1	- 144.00000 *	26.412 12	.003	-211.8945	-76.1055
			Sample 3	22.00000	29.529 65	.490	-53.9084	97.9084
		Sample 3	Sample 1	- 166.00000 *	29.529 65	.002	-241.9084	-90.0916
			Sample 2	-22.00000	29.529 65	.490	-97.9084	53.9084
GPx	LSD	Sample 1	Sample 2	-.06067	3.7228 7	.987	-8.4824	8.3610
			Sample 3	9.53350*	3.4196 7	.021	1.7977	17.2693
		Sample 2	Sample 1	.06067	3.7228 7	.987	-8.3610	8.4824
			Sample 3	9.59417*	3.8934 7	.036	.7865	18.4018

		Sample 3	Sample 1	-9.53350*	3.4196 7	.021	-17.2693	-1.7977
			Sample 2	-9.59417*	3.8934 7	.036	-18.4018	-.7865
GGT	LSD	Sample 1	Sample 2	-5.79267*	.89210	.001	-7.9756	-3.6098
			Sample 3	-3.86067*	.89210	.005	-6.0436	-1.6778
		Sample 2	Sample 1	5.79267*	.89210	.001	3.6098	7.9756
			Sample 3	1.93200	.89210	.073	-.2509	4.1149
		Sample 3	Sample 1	3.86067*	.89210	.005	1.6778	6.0436
			Sample 2	-1.93200	.89210	.073	-4.1149	.2509

*. The mean difference is significant at the 0.05 level.

LIPASE

	SAMP	N	Subset for alpha = 0.05	
			1	2
Tukey B ^{a,b}	Sample 3	2	102.000 0	268.000 0
	Sample 2	3	124.000 0	
	Sample 1	3		
Duncan ^a ,b	Sample 3	2	102.000 0	268.000 0
	Sample 2	3	124.000 0	
	Sample 1	3		
	Sig.		.475	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.571.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

GPx

	SAMP	N	Subset for alpha = 0.05	
			1	2
Tukey B ^{a,b}	Sample 3	4	26.0525	
	Sample 1	5	35.5860	
	Sample 2	3	35.6467	
Duncan ^a ,b	Sample 3	4	26.0525	
	Sample 1	5		35.5860

Sample	3		35.6467
2			
Sig.		1.000	.987

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 3.830.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

GGT

	SAMP		Subset for alpha = 0.05	
	LE	N	1	2
Tukey	Sample	3	1.9300	
B ^a	1			
	Sample	3		5.7907
	3			

	Sample 2	3		7.7227
Dunca n ^a	Sample 1	3	1.9300	
	Sample 3	3		5.7907
	Sample 2	3		7.7227
	Sig.		1.000	.073

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.